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Potential Use of Long Noncoding RNAs as Biomarkers for Astrocytoma

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Abstract

Noncoding RNAs represent a high proportion of the human genome and regulate gene expression by means of innumerable and unimaginable modes of action. Particularly, long noncoding RNAs have emerged as central regulators of gene expression and alterations on their function have been associated with many types of cancer, such as astrocytomas. Astrocytomas are the most common type of gliomas in the central nervous system, and glioblastoma multiforme is their most aggressive form. Although adult and pediatric astrocytomas exhibit certain molecular similarities, they are considered as distinct molecular entities. Since to date there is no effective treatments for these tumors, different efforts are being made to find molecular tools useful for this purpose. Studies have shown that both tumor and circulating expression of lncRNAs were altered in astrocytoma, which was useful to distinguish the patients with this neoplasia from those without cancer, as well as to determine different prognostic factors related to the disease. According to these studies, different “molecular signatures” of specific lncRNAs were established, and they have a potential use in the medical practice. From a system biological perspective, complex interaction networks, conformed by lncRNAs, microRNAs, mRNAs, and proteins, were elucidated and predicted to control many oncogenic processes.

Keywords: astrocytoma, biomarker, interacting network, lncRNA, microRNA

1. Introduction

Noncoding RNAs (ncRNAs) represent a significant fraction of the human genome [1], and the great diversity and forms of action of these RNA species has put them at the center of biomedical research of diseases, such as cancer [2–4]. lncRNAs are not the exception, and many of them have been proposed as possible diagnostic and prognostic biomarkers for Ast [5]. lncRNAs are RNAs of more than >200 nucleotides in length, which have to meet certain additional criteria to be classified within this category [6]. The evolutionary conservation of lncRNAs among species is poor [7], and they are transcribed by a variety

of transcriptional mechanisms [6]. Many cellular processes are regulated by lncRNAs and this could be at both cytoplasmic and nuclear levels, as well as distance by moving them to their target tissues through different bodily fluids, such as blood [8]. LncRNAs exert their functions by establishing interactions with other lncRNA and RNA species, as well as with proteins [9], and changes on their functioning have been associated with cancer and particularly with astrocytomas (Ast) [5].

Gliomas represent 81% of the Central Nervous System (CNS) tumors of which the most common subtypes in adults are glioblastoma multiforme (GBM), anaplastic Ast (AAst), and oligodendrogliomas [10, 11]. In the pediatric counterpart, pilocytic Ast (PAst) is the most common type in pediatric age [11]. According to the new classification of the World Health Organization (WHO), Ast are now classified according to the presence or absence of *IDH1/IDH2* mutations, as well as by phenotypic traits and integral diagnoses [12]. Those tumors with *IDH1/IDH2* mutations were classified as “diffuse gliomas,” a new group that includes diffuse Ast (DAst; Grade II), AAst (Grade III), GBM, and diffuse oligodendrogliomas (Grade I and II) [12]. Meanwhile, pilocytic Ast (PAst; Grade I), subependymal giant cells Ast (Grade I), and pleomorphic xanthoastrocytoma (Grade II) were excluded from the diffuse group, given that they do not have these mutations [12]. Although there are certain molecular similarities between adult and pediatric Ast (p-Ast) [13], their molecular differences are well established and based on this, they are classified as different tumor subtypes [14–17]. Although there have been advances in the Ast study—mainly on adult GBM—, to date, there are very few molecular tools useful for Ast diagnosis, prognosis, and treatment. Essentially, most studies have identified changes on the expression of lncRNAs in both tumor tissues and GBM cell lines, and according to this, some “molecular signatures” have been postulated for the diagnosis and prognosis of Ast. For instance, circulating lncRNAs have allowed the distinction of patients sensitive or resistant to treatments, specifically to temozolomide (TMZ) or radiotherapy [18, 19]. In addition, the establishment of bioinformatic algorithms identified interactome networks in which lncRNAs physically interact with other lncRNAs, as well as with messenger RNAs (mRNAs) and microRNAs (miRNAs), and proteins. These studies have shown that expression changes of lncRNAs could lead to the amplification of the aberrant signals, which in turn could lead to alterations of many signaling pathways and cellular processes [5, 20, 21]. In p-Ast, high expression levels of LINC-ROR (long intergenic nonprotein coding RNA, regulator of reprogramming) were useful to distinguish p-Ast from the control, as well as to identify the GBM from the rest of the Ast grades; this strongly suggests the involvement of LINC-ROR in p-Ast diagnosis and prognosis [5].

2. Astrocytoma

Although the new WHO classification of tumors of the CNS takes into account phenotypic traits, it also takes into account other criteria, such as the genotype and integral diagnoses of the disease [12]. According to this classification, Ast are now classified mainly by the presence or absence of *IDH1/IDH2* mutations and based on this, diffuse Ast (DAst; Grade II) and anaplastic Ast (AAst; Grade III), as well as the GBM, and diffuse oligodendrogliomas (Grade I and II) were classified as “diffuse gliomas” [12]. PAst, subependymal giant cells Ast, and pleomorphic xanthoastrocytoma (Grade II) were classified in a different group, because of the absence of *IDH1/IDH2* mutations.

2.1 Astrocytomas that lack IDH1 and IDH2 mutations

These tumors have a well circumscribed growth pattern, lack IDH alterations, and they frequently have *BRAF* (pilocytic Ast (Past) and pleomorphic xanthoastrocytoma) and *TSC1/TSC2* mutations (subependymal giant cells Ast) (**Table 1**).

PAst are the most common type of Ast in pediatric age and are characterized by their biphasic pattern: compact bipolar cells with Rosenthal fibers, microquistes, and granular bodies (**Figure 1A**). As a general rule, PAst are well-defined tumors

WHO Classification		
Astrocytomas that lack IDH1 and IDH2 mutations		
Pilocytic astrocytoma	}	WHO grade I
Subependymal giant cell astrocytoma		
Pleomorphic xanthoastrocytoma	}	WHO grade II
Diffuse gliomas		
Diffuse astrocytoma, IDH-mutant	}	WHO grade II
Gemistocytic astrocytoma, IDH-mutant		
Diffuse astrocytoma, IDH-wildtype		
Diffuse astrocytoma, NOS		
Anaplastic astrocytoma, IDH-mutant	}	WHO grade III
Anaplastic astrocytoma, IDH-wild type		
Anaplastic astrocytoma, NOS		
Anaplastic pleomorphic xanthoastrocytoma		
Glioblastoma		
GBM, IDH-mutant		
GBM, IDH-mutant		
GBM, NOS		
GBM Variants		
Epithelioid glioblastoma		
GBM with primitive neuronal component		
Small cell GBM/Ast		
Granular cell GBM/Ast		

Table 1.
Astrocytoma classification according to the World Health Organization (2016).

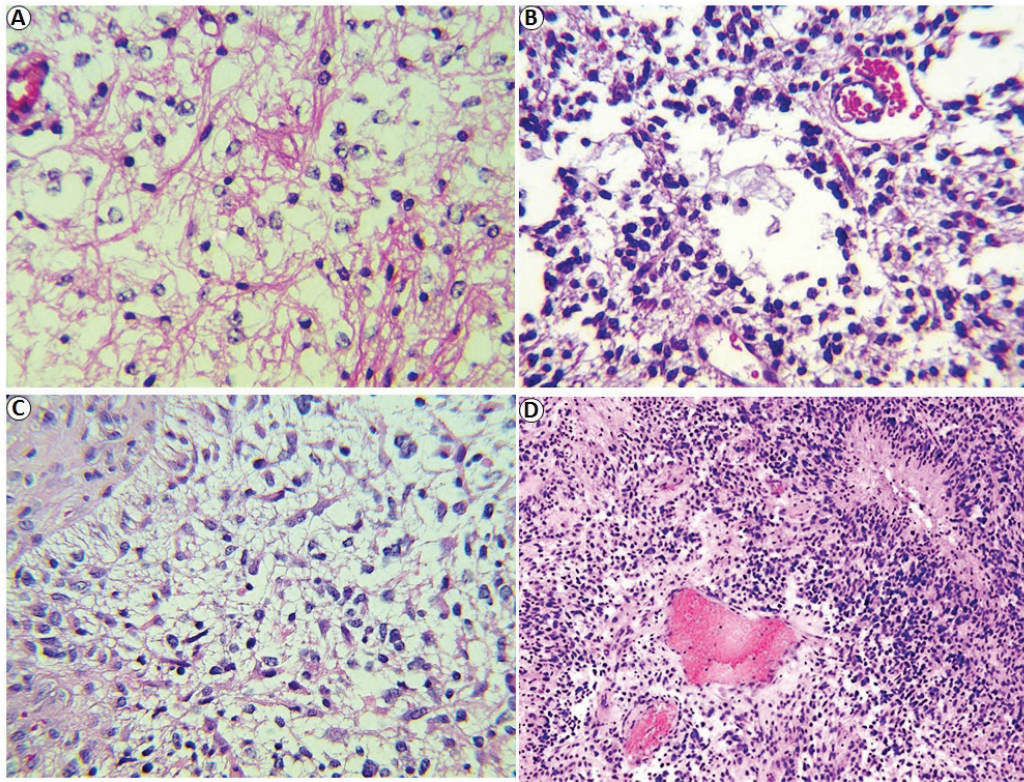


Figure 1.

(A) Pilocytic Ast. Photomicrograph that shows a glioma with astrocytes, which have an elongated cytoplasm and a pilocytic appearance, on a loose stroma (H&E, 40×). (B) Diffuse astrocytoma. Diffuse glioma with cysts and solid areas; the cells are homogenous and do not exhibit atypia (H&E, 40×). (C) Anaplastic astrocytoma. Neoplasia with hypercellularity and nuclear pleomorphism and hyperchromatism. In the upper left, a blood vessel with a glomerular pattern can be seen (H&E, 40×). (D) GBM. Hypercellular glial tumor with diffuse pleomorphism and necrosis; it is delimited by palisaded cells, which are characteristic of GBM (H&E, 10×). Photomicrographs taken at the pediatric Pathology Service of the Children's Hospital, National Medical Center Century XXI, IMSS.

(**Figure 2A**); so they can be surgically resected without causing damage to the adjacent tissue and they do not progress to more aggressive stages; therefore, PAs are considered as neoplasms of good prognosis.

PAs are developed along the neuroaxis, and they are preferably located in the cerebellum [22–24]. It is important to mention that there are genetic diseases such as neurofibromatosis 1 (*NF-1*), which influences the formation of PAs; approximately 15% of individuals with *NF-1* develop these type of tumors, specifically at the level of the optic nerve [25, 26].

2.2 Diffuse gliomas (tumors with IDH1 and IDH2 mutations)

In the previous WHO classification, diffuse Ast (DAst) were classified as an independent group, but now they are classified along with anaplastic Ast (Aast; Grade III) and glioblastoma (GBM; Grade IV) (**Figures 1B–D** and **2B–D**), as well as with diffuse oligodendrogliomas (Grade I and II) [12]. Although factors such as growth and tumor behavior are still taken into account, the feature that distinguishes them as diffuse gliomas are the *IDH1* and *IDH2* mutations; however, these tumors can be subclassified into the *IDH*-mutant, *IDH*-wildtype, and NOS categories [12].

IDH-wildtype neoplasms constitute a subgroup of uncommon tumors, which are negative for mutant R132H *IDH1* protein and genic mutations for *IDH1* (codon 132) and *IDH2* (codon 172). Importantly, DAst (WHO Grade II) and AAst (WHO Grade III) can be confused with gangliogliomas and *IDH*-wildtype GBM [27, 28].

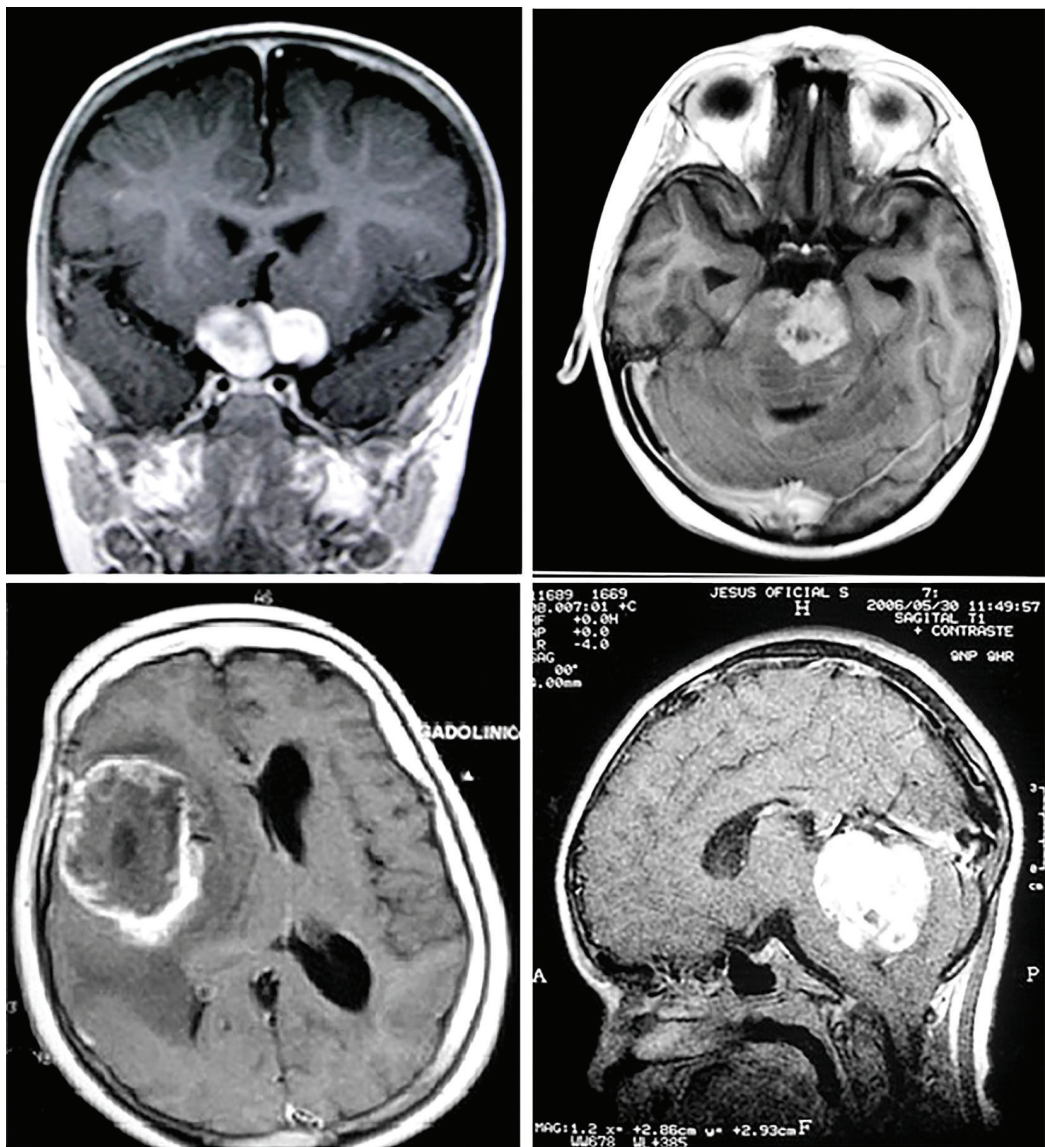


Figure 2.
(A and B) Pilocytic and diffuse astrocytoma. These tumors are both isodense and hypodense to the brain and show calcifications in 15–20% of cases, and they have virtually no edema. (C) Anaplastic astrocytoma is poorly defined lesions with heterogenous signal strengths. Mixed areas of isodensity to hipodensity are observed; these tumors may have hemorrhagic foci. It is common to observe a hypertensive central nucleus, which is surrounded by an intense edge with peripheral finger-like projections and secondary to a vasogenic edema. (D) GBM. Heterogenous lesion with cellular components of mixed signal, central necrosis, and hemorrhage; calcifications are rare.

Tumors that do not have any of these molecular tests—immunohistochemistry or sequencing—are subclassified as DAst-NOS or AAst-NOS, respectively [12].

2.2.1 Glioblastoma

According to the new WHO classification, the GBM was also classified into the group of diffuse gliomas and subclassified into the *IDH*-mutant or *IDH*-wildtype categories, or the NOS category. The *IDH*-wildtype form represents ~90% of cases and was associated with primary GBM (*de novo*), which are more common in patients older than 55 years of age [29]. Meanwhile, the *IDH*-mutant GBMs (~10%) are tumors that develop from low-grade diffuse glioma and are commonly present in younger patients; this type of GBM are also known as secondary GBM [29]. Similar to that described above, GBM-NOS are those tumors that do not have a full *IDH* evaluation [12]. According to phenotypic traits and the genetic background, to date, there are different GBM variants (**Table 1**).

2.3 Pediatric diffuse gliomas

Pediatric diffuse gliomas have the K27 mutation in the gene *H3F3A* (H3 Histone Family Member 3A) and less commonly in the related gene *HIST1H3B* (Histone Cluster 1 H3 Family Member B). Although they are mainly present in children, they can also be present in adults. These tumors exhibit a diffuse growth pattern and a midline location: thalamus, brain stem, and spinal cord; therefore, they are classified as diffuse midline glioma, H3 K27 mutant, and include tumors previously known as diffuse intrinsic pontine glioma (DIPG) [12].

3. LncRNAs in astrocytoma

LncRNAs have emerged as important molecular elements in different types of cancer, and Ast are not the exception [5, 30–32]. To date, diverse studies have shown the high complexity of the lncRNA study in Ast, due to the wide variety of mechanisms by which lncRNAs exerts their biological actions and because of the high tumor heterogeneity [33–35]. Changes in the nucleotide sequence of lncRNAs, their transcription rate, the expression of specific variants, in their expression levels, among others, could lead to an aberrant amplification of cell signals [36–38]. Given that GBM is the most aggressive type of cancer that begins within the brain [39, 40], most studies have been focused on this tumor subtype and to a lesser extent in the other WHO grades of adult Ast or in all WHO grades of p-Ast. Despite the significant effort that has been made in recent years to learn more about Ast, to the best of our knowledge, to date, there are very few molecular tools really applicable to diagnose, prognose, or treatment of these tumors [41–43]. Therefore, there is great interest to establish these molecular tools for GBM and evidence indicates that lncRNAs seem to be good candidates to serve such purpose.

3.1 LncRNAs as potential astrocytoma biomarkers

Expression changes of a biomolecule are a powerful tool to establish molecular “signatures” or “fingerprints” useful to distinguish and identify subgroups of a disease with a particular clinical behavior [44–47]. In this sense, expression changes of lncRNAs have been useful to differentiate both adult and pediatric Ast from non-neoplastic tissues, and some of them have the potential to be used in the medical practice as biomarkers. The meta-analysis performed by Zhang et al. [48] demonstrated for the first time the usefulness of the lncRNAs aberrantly expressed for Ast diagnosis and prognosis. This study showed that the expression profile of lncRNAs allowed to differentiate Ast or oligodendrogliomas from nonneoplastic tissues and to associate it with Ast malignancy or with lineage distinction in gliomas (**Table 2**). Subsequently, the same group established the first “molecular signature” of lncRNAs for Ast diagnosis and prognosis, which distinguished this neoplasia from nonneoplastic tissues, as well as Ast malignancy or patient’s survival (**Table 2**) [49]. Additionally, a second group of precise lncRNAs was specific for Ast, and it was functional to differentiate them from the control tissues; from this signature, two lncRNAs were also associated with Ast malignancy, since their expression distinguished Ast WHO grades (**Table 2**) [50]. However, none of the lncRNAs that were part of the first molecular signature was established in the second, which could be related to the samples included in each study—referring to age, sex, with or without treatment, radiotherapy, among others—, as well as to the bioinformatic approach used in each study. This evidence emphasizes the importance that has the

Molecular signature (possible biomarkers for Ast diagnosis)	LncRNAs associated with Ast malignancy (possible biomarkers for Ast prognosis)	LncRNAs associated with patient's survival (possible biomarkers for prognosis)
Zhang et al. 2012; 2013		
KIAA0495 PART1 MGC21881 MIAT GAS5 PAR5 Up-regulated	CRNDE-A CRNDE-B HOTAIRM1 Up-regulated RP11-361F15.2-A RP11-361F15.2-B RFPL1S NCRNA00086 C21orf131-A Down-regulated	KIAA0495 Poor prognosis PART1 MGC21881 MIAT GAS5 PAR5 Better prognosis
Zhi et al. 2015		
ENST00000244906 ENST00000545440 NR_002809 ENST00000436616 Up-regulated XLOC_010967 BC002811 ASO1937 Down-regulated	ENST00000545440 NR_002809	NR_002809 BC002811 XLOC_010967 Poor prognosis
Matjasic et al. 2017		
LOC285758 Up-regulated/ hypomethylated	LOC285758 gradual increase in its expression from grade I to GBM.	
Xu et al. 2017		
		lnc-C19orf57-6:6 lnc-ALYREF-1:1 SNHG1 Poor prognosis

Table 2.
Molecular signatures of lncRNAs in astrocytoma.

homogenization of patient’s samples included in a study has and how crucial it is to specify the clinic features of the included patients.

In addition to changes in the lncRNA expression, their promoter methylation status seems to be useful for Ast diagnosis and prognosis. Specifically, it was shown that expression and the promoter methylation pattern of *LOC285758* (Long Intergenic Non-Protein Coding RNA 1268) differentiate all Ast WHO grades and other gliomas (oligodendroglioma and oligoastrocitoma (II–III)) from the control, as well as Ast grades I–III from both primary and secondary GBM (**Table 2**) [32]. Based on this evidence, the identification of the mechanisms that lead to an aberrant expression of the lncRNAs—such as epigenetic regulation—could be part of the biomarkers package useful in the medical practice.

3.2 LncRNAs as potential GBM biomarkers

Specifically for GBM, different lncRNAs have also been found as potential biomarkers for its diagnosis and prognosis (**Table 3**). In this sense, Xu et al. [51] identified lncRNAs, which were associated with patient’s survival; particularly, high expression of *SNHG1* (small nucleolar RNA host gene 1) was related with poor prognosis (**Table 3**). Meanwhile, *in silico* analysis showed many differentially expressed lncRNAs useful to distinguish GBM from nonneoplastic tissues and each

Diagnosis	<div>CRNDE CYTOR</div> <div>TUNAR LINCO1476</div> <div>Up-regulated</div> <div>Down-regulated</div>
Prognosis	<div>CRNDE</div> <div>SNHG1</div> <div>RP11-334C17.6</div> <div>BTAT10</div> <div>Circulating lncRNAs</div> <div>HOTAIR GAS5</div> <div>MALAT1</div> <div>Poor prognosis</div> <div>Poor prognosis</div> <div>High expression Low expression</div> <div>High expression Low expression</div> <div>Higher median survival times Lower median survival times</div> <div>Lower median survival times Higher median survival times</div> <div>Up-regulated</div> <div>High expression</div> <div>Survival and GBM progression</div> <div>Poor overall survival High recurrence of the disease</div>
TMZ resistance	<div>RP11-838N2.4 MALAT1 H19</div> <div>Up-regulated</div>

Table 3.
LncRNAs as potential GBM biomarkers.

of the four GBM subtypes: classical, mesenchymal, neural, and proneural. The lncRNAs *CRNDE* (colorectal neoplasia differentially expressed) and *CYTOR* (cytoskeleton regulator RNA) (both upregulated) and *TUNAR* (TCL1 upstream neural differentiation-associated RNA) and *LINCO1476* (both downregulated) were those with the highest expression changes in GBM compared to the control and with a potential use for GBM diagnosis (Table 3) [52]. *CRNDE* overexpression has been associated with high cell proliferation, migration, and invasion, which corresponds with the promotion of tumor growth observed in *in vivo* studies [53]. In addition, the expression pattern of *RP11-334C17.6* and *BTA10* allows to group patients with a greater survival from those with worse results, as well as the prognosis of each of the four GBM subtypes [52]. Currently, the available data are promising, and based on them, specific lncRNAs have been postulated as potential biomarkers for GBM diagnosis and prognosis, which with further evidence could be used in the medical practice.

3.3 Circulating lncRNAs

It is a fact that the establishment of novel biomarkers for Ast is essential and their identification and clinical application by means of less invasive methods

would be ideal. To date, many studies have demonstrated the usefulness of circulating lncRNAs for diagnosis and prognosis of many diseases, including GBM [46, 54, 55]. The profile expression of lncRNAs was determined in blood serum of GBM patients and high levels of *HOTAIR* (HOX transcript antisense RNA) and *GAS5* [growth arrest specific 5 (nonprotein coding)] were prognostic factors to determine patient's survival and GBM progression [56]. Overexpression of circulating *HOTAIR* has been observed in different types of cancer [57–61], but its downregulation was detected in patients with acute myocardial infarction [62]. Interestingly, the presence of high levels of circulating *HOTAIR* DNA was also detected in breast cancer (BC) patients, where it has a potential use for BC diagnosis [60]. Unlike those observed in the GBM, most studies have shown that circulating *GAS5* was downexpressed in different types of cancer, which allowed the diagnosis of both intraductal papillary mucinous neoplasms [63] and nonsmall cell lung cancer [64, 65], as well as BC prognosis [66]. By contrast, overexpression of circulating *GAS5* could be used to predict treatment response in head and neck cancer [67]. According to this, the overexpression of *HOTAIR* observed in all cancer types studied to date strongly suggests a central function of this lncRNA in the establishment, maintenance, and/or progression of cancer in general. Therefore, it is very important to identify the processes that *HOTAIR* is controlling in cancer in order to postulate molecular tools to eradicate neoplastic cells.

MALAT1 [metastasis associated lung adenocarcinoma transcript 1 (nonprotein coding)] was another lncRNA with changes on its circulating expression levels in GBM. This lncRNA was overexpressed, and this was associated with poor overall survival and with a high GBM recurrence [19]. Overexpression of circulating *MALAT1* has been observed in many types of cancer and it seems to be useful for cancer diagnosis and prognosis [68–73]. On the contrary, Peng et al. [74] showed that *MALAT1* downregulation in blood was important for early diagnosis in nonsmall cell lung cancer. Based on the above, the presence of a biomolecule in distinct corporal fluids is a noninvasive form at the molecular level either by the presence or the absence of a disease, as well as by the patient's prognosis with an specific disease. According to the studies performed to date, the use of circulating lncRNAs in the medical practice seems very promising.

3.4 Search for GBM biomarkers from a system biological perspective

Since a biomolecule does not act alone and depends on the cellular context to carry out its biological functions, different groups of study have focused on the identification of the lncRNA interactome in GBM. Evidence indicates that lncRNAs could interact with themselves, as well as with other biomolecules, such as mRNAs, miRNAs, and proteins; changes on the lncRNA activity at distinct molecular levels could affect their interaction networks and the correct cellular functioning [5, 75–77].

Yan et al. [78] established interaction networks between lncRNAs and mRNAs aberrantly expressed in GBM, and based on this, they postulated “hub genes” which were involved in GBM pathogenesis. Similarly, under this perspective, it was found that complexes conformed by lncRNA•mRNA (*HOTAIR-MX11-CD58/PRKCE* and *HOTAIR-ATF5-NCAM1*) or lncRNA•lncRNA (*MCM3AP-AS-MIR17HG*) could be potential biomarkers for GBM prognosis [79–82]. Importantly, the *TP73-AS1•RFX1* complex (TP73 Antisense RNA 1 and Regulatory Factor X1, respectively) was identified as an important factor for the control of apoptosis in this type of tumor [83]. To sum up, the cancer study from a system biological perspective has allowed to identify the complex interaction networks where many biomolecules are involved

to regulate specific cellular processes; alterations in the operation of any of these components will affect the correct functioning of the cell. Specifically, lncRNA changes could lead to an amplification of the aberrant signals and this could be more significant if the lncRNAs interact with other ncRNAs, given that they have many targets of regulation.

3.5 Radio and chemoresistance

A major clinical problem is the resistance to chemotherapy and radiotherapy; therefore, identification of “molecular tools” that can predict and in the best-case scenario, improve the cellular response to these treatments would be ideal. Wang et al. [80] established a prediction model for radiosensitivity by detecting differentially expressed lncRNAs and mRNAs after irradiation. Interestingly, the algorithm differentiated those patients that were radiosensitive and with a greater survival, from the patients with radioresistance; unfortunately, as far as we know, this is the only study focused on GBM radioresistance.

In addition, the involvement of lncRNAs in chemoresistance has been widely studied. lncRNAs *RP11-838 N2.4* [84] and *MALAT1* [19, 55] were shown to be associated with TMZ resistance (**Table 3**). HiSeq sequencing identified the profile expression of lncRNAs, which was specific and differentiated patient resistant to TMZ from those sensitive to this drug. This analysis showed that overexpression of *MALAT1* and its circulating form was related to a lower response to chemotherapy and to a shorter survival time of patients with GBM by controlling the miR-203 and *TYMS* (thymidylate synthase) levels, which was tested in TMZ resistant GBM cells [19]. Another fact worthy of mention is that other components of the *MALAT1* interactome have been elucidated to be important for TMZ resistance. *MALAT1* overexpression maintained high levels of expression of specific genes, such as *ABCB1* (ATP binding cassette subfamily B member 1), *ABCC5* (ATP binding cassette subfamily C member 5), *LRP1* (LDL receptor related protein 1), and *ZEB1* (zinc finger E-box binding homeobox). Notably, forced decrease of *MALAT1* resulted in TMZ sensitization by decreasing the levels of *ZEB1* [55]. Meanwhile, alterations in the axis *RP11-838 N2.4*•miR-10•EphA8 (EPH Receptor A8) were also involved in GBM cell resistance to TMZ [21]. All these facts supported the importance of the study of lncRNAs for clinical purposes and specifically gain knowledge regarding the prognosis of patients to radiotherapy or chemotherapy.

3.6 LncRNAs in stem cells

Many lines of evidence have shown the involvement of lncRNAs in the control of many cellular processes in cancer stem cells (CSCs) [85–87], but their participation in Ast has been very poorly studied. These cells are able to self-renew and differentiate into diverse cancer cell lineages to form tumors, so CSCs have been proposed as potential targets for cancer treatment. To further understand this, Balci et al. [88] determined the profile expression of lncRNAs in GBM stem cells (GSCs) relative to control stem cells. From these differentially expressed lncRNAs, *PCAT-1* (prostate cancer associated transcript 1 (nonprotein coding)), *MEG3* (maternally expressed 3 (nonprotein coding)), and *HOTAIR* functioned as tumor suppressors in GBM. This was related to alterations in gene expression. Interestingly, another study identified that even identical GSCs showed variations in their expression profile of lncRNAs, as well as in the variants produced by specific subgroups of cells. Despite this, the authors could establish a stem cell

signature of 31 lncRNAs according to their expression levels [57]. Meanwhile, in hypoxic conditions, the expression of the lncRNA *HIF1A-AS2* (hypoxia inducible factor 1 alpha-antisense RNA 2) was induced and this led to positive control of the growth, self-renewal, and molecular reprogramming of the GSCs [20]. Significantly, the control of these cellular processes was possible by regulating an interaction network, which will be described later.

Although many studies have focused on studying the changes on the expression of lncRNAs, very few have attempted to determine the mechanisms underlying this deregulation. In this sense, Zhang et al. [89] showed a feedback loop which controlled the expression of the lncRNA *FOXM1-AS* (Forkhead box M1 antisense) and it proved to be important for GSC tumorigenesis. *ALKBH5* (AlkB homolog 5, RNA demethylase) is a demethylase highly expressed in GBM GSCs, which was associated with an enhanced self-renewal and tumorigenesis of these cells. These malignant cell processes were controlled by *FOXM1* (Forkhead box M1) and *FOXM1-AS*, which increased their expression levels by a greater demethylation of the immature transcripts of *FOXM1*. In this pathway of regulation, *FOXM1-AS* was important to facilitate the action of *ALKBH5* on the nascent transcripts of *FOXM1*; therefore, a therapy in which the action of this lncRNA was reduced or blocked could be important to prevent GBM tumorigenesis. Taken together, these studies showed that although expression changes of lncRNAs were useful for GBM diagnosis and prognosis, they necessarily not represent the entire tumor, but rather this seems to associate with certain subgroups of cells that predominate over others and express particular lncRNAs. Therefore, the applicability of a differentially expressed biomolecule in the medical practice—particularly lncRNAs—must be done with caution and with all the required evidence.

4. Action mechanisms of lncRNAs in GBM

In addition to expression changes, it is necessary for the elucidation of the action mechanisms by which lncRNAs are acting. Evidence showed that lncRNAs act at both cytoplasmic and nuclear levels and that this is done directly and/or by their interaction with protein complexes and/or with other lncRNAs or different RNA species, such as mRNAs and miRNAs [5, 75–77]. Also, lncRNAs can regulate many signaling pathways by controlling the cytoplasmic disposal of mRNAs and miRNAs and even by producing small RNA species, such as miRNAs [89].

4.1 Sponge lncRNAs

This class of lncRNAs regulates miRNA disposal in the cell cytoplasm by capturing them and blocking their action [90, 91]. To date, all lncRNAs identified as “sponges” in the GBM acting as suppressors and involved in lncRNA upregulation and miRNA attenuation were associated with GBM **Table 4**). LncRNAs *H19* (imprinted maternally expressed transcript (nonprotein coding)) and *NEAT1* (nuclear paraspeckle assembly transcript 1 (nonprotein coding)) controlled the action of the miRNA let-7e, whose levels were downregulated in GBM due to the overexpression of these lncRNAs [92, 93]. Specifically, the axis *H19*•let-7e was involved in maintaining the phenotype of stem cells, which was associated with tumor malignancy and TMZ chemoresistance [93]. Similarly, a low disposal of let-7e by *NEAT1* overexpression, resulted in a higher activity of its mRNA target *NRAS* (*NRAS* proto-oncogene, GTPase), which leads to GBM malignancy [92].

"Sponges" LncRNAs	microRNA	mRNA target	Cellular process altered	Signaling pathway
<i>Tumor suppressors</i>				
H19 NEAT1	Let-7e	NRAS (NRAS Proto- Oncogene, GTPase)	H19: stem cells phenotype NEAT1:	
XIST	miR-152		Proliferation, migration, invasion, apoptosis evasion, tumor growth and poor mice survival	
TUG1	miR-299	VEGFA (Vascular Endothelial Growth Factor A)	Angiogenesis induction	
RP11-838N2.4	miR-10	EphA8 (EPH Receptor A8)	Apoptosis evasion	Apoptosis
SNHG7	miR-5095	CTNNB1 (Catenin Beta 1)	Proliferation, migration, invasion, apoptosis evasion	Wnt/ β catenin
MALAT1	miR-203	TYMS (Thymidylate Synthetase)	Low chemotherapy response Shorter survival time of patients	
CRNDE	miR-136-5p	Wnt2 (Wnt Family Member A2) BCL2 (BCL2 Apoptosis Regulator)	Apoptosis evasion	Wnt Apoptosis
CASC2	miR-101	CPEB1 (Cytoplasmic Polyadenylation Element Binding Protein 1)	Cell proliferation Tumorigenesis	

Table 4.
LncRNAs as sponges in adult GBM.

Other lncRNAs that function as sponges in GBM were related to tumor malignancy. For example, the upregulation of *XIST* (X inactive specific transcript (nonprotein coding)) was related to GSC malignancy, tumor growth, and poor mice survival by controlling the action of miR-152 [94]. Meanwhile, the attenuation of the miR-299 disposal was controlled by the overexpression of *TUG1* (lncRNA taurine upregulated 1), which was related to tumor malignancy by the overactivation of *VEGFA* (vascular endothelial growth factor A) [95] and apoptosis evasion [93].

Similarly, GBM malignancy was mediated by the overexpression of *RP11-838N2.4* and *SNHG7* (small nucleolar RNA host gene 7), which regulated the function of miR-10 and miR-5095, respectively. In the first case, the attenuation of the action of miR-10 was associated with apoptosis evasion, and the reestablishment of the axis *RP11-838 N2.4*•miR-10•*EphA8* (EPH receptor A8) induced this programmed cell death [21]. Meanwhile, reestablishment of the *SNHG7*•miR-5095•*CTNNB1* (catenin beta 1) axis arrested tumor growth and decreased metastasis by decreasing the expression of *CTNNB1*, which is involved in the Wnt/ β -catenin pathway [96]. Finally, it was observed that GBM proliferation, migration, and invasion were also promoted by the overexpression of *CRNDE* and the consequent attenuation of the miR-136-5p expression; all these led to the overactivation of *BCL2* and *WNT2*, which are target genes of this miRNA [97]. According to the LNCipedia compendium, there are many variants reported for these lncRNAs; therefore, it would be very interesting and important to identify which lncRNA

variants are expressed in GBM and which of them have the binding sites for trapping these miRNAs. Also, further studies are necessary to know if *H19* and *NEAT1* regulate the action of let-7e in a synergistic manner.

A very interesting case was that of the lncRNA *CASC2c* (cancer susceptibility candidate 2; formerly C10orf5). Besides its interaction with miR-101, this lncRNA was involved in the processing of the pre-miR-101 into mature miR-101 and competed with this miRNA for the mRNA *CPEB1* (cytoplasmic polyadenylation element binding protein 1). High levels of *CASC2c* and consequently a reduced activity of the axis miR-101•*CPEB1* were associated with a high cell proliferation and tumorigenesis. Therefore, a decrease in *CASC2c* expression and an increased disposal of miR-101 were related to better patient's prognosis [98]. This evidence is an indication of all biological functions that an lncRNA can play in the cell and how the system ensures the regulation of gene expression by regulating at different levels the biogenesis of miRNAs (**Figure 3**). In consequence, if something modifies the processing of the pre-miR-101 or affects the regulation of its mature form, *CASC2c* would try to compensate the miRNA action by competing for its target genes. Evidently, other mechanisms must be involved in the biogenesis of this miRNA.

4.2 By interacting with mRNAs

Besides the lncRNA interaction with miRNAs, there is evidence indicating that lncRNAs can carry out their biological functions when they interact with mRNAs and/or proteins [5, 75–77]. As mentioned above, *HIF1A-AS2* was involved in the GSC malignancy under hypoxia conditions. The action of this lncRNA was performed in part by directly interacting with *IGF2BP2* (insulin-like growth factor 2 mRNA binding protein 2) and *DHX9* (DEXH-box helicase 9), which finally controlled the action of *HMGA1* (high mobility group AT-Hook 1) [20]. According to this, elucidation of all the components that formed the interactome network of *HIF1A-AS2* in the GSCs would be crucial to establish molecular tools for GBM treatment.

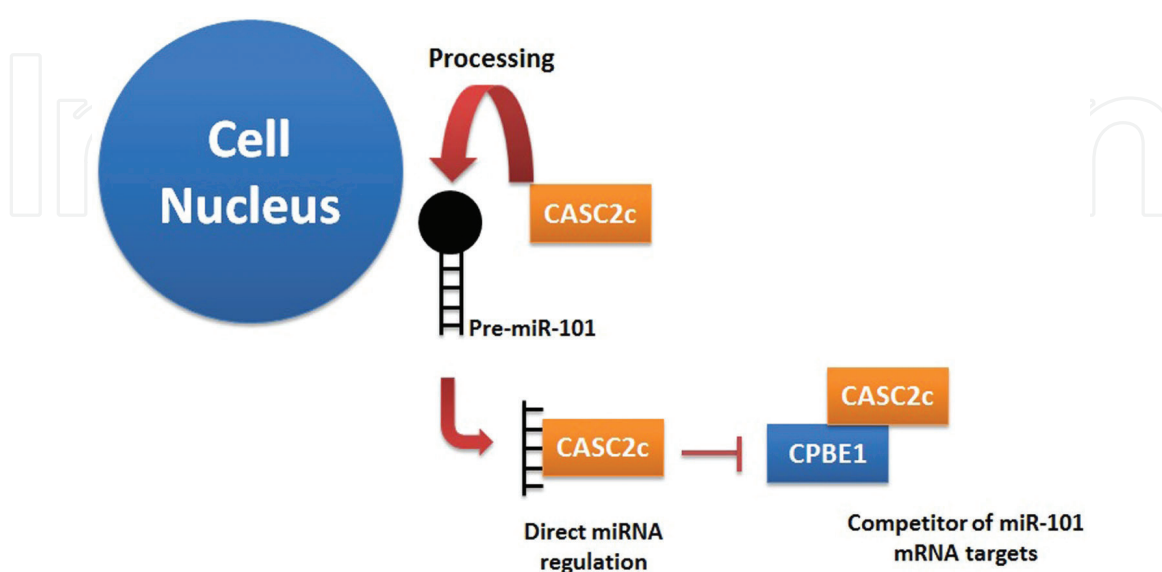


Figure 3.
 The lncRNA *CASC2* regulated the function of the miR-101 at different molecular levels. *CASC2* was involved in the processing of the pre-miR-101 and also interact with its mature form to regulate the function of this miRNA. If any of these mechanisms fail, *CASC2* ensures the miR-101 regulation by interacting with its mRNA targets.

5. Pediatric Ast

Adult and p-Ast are distinct molecular entities and are classified into different groups; therefore, studies in pediatric Ast are imperative. The first study performed in p-Ast was the one where the overexpression *HOTAIR* and *HOX* was detected in different pediatric brain tumors, including juvenile pediatric Ast (JPA); however, the biological meaning of this was not further studied [99].

We identified in the laboratory the expression profile of lncRNAs in p-Ast of WHO grades I–IV, given that the function of lncRNAs in p-Ast has been poorly studied. Similar to that observed for adult Ast, p-Ast showed many lncRNAs with expression changes relative to the control tissues, among histological grades or even in the same histological grade [5]. In addition, it was identified that the interaction of many differentially expressed lncRNAs with mRNAs and/or miRNAs aberrantly expressed was identified. As explained above, these interactions could lead to the amplification of the aberrant signals and to the modification of many signaling pathways. According to this, there were several hub lncRNAs in p-Ast that in relation to their interactions with mRNAs could be altering pathways such as FOXO, chemokine, hedgehog, MAPK, and others (Figure 4). Additionally, hub lncRNAs potentially useful to distinguish GBM from the other histopathological WHO grades were predicted to control diverse metabolic pathways and signaling pathways such as Ras, hippo, apellin, etc. (Figure 4).

The interaction of differentially expressed lncRNAs and miRNAs was shown to be a complex network that could be involved in modifications on proteoglycans in cancer, fatty acid metabolism, cell cycle, and spliceosome. Notably, data analysis revealed the presence of circular lncRNAs (circRNAs) with expression changes in p-Ast (Figure 5). According to the interactions of circRNAs with miRNAs, this type

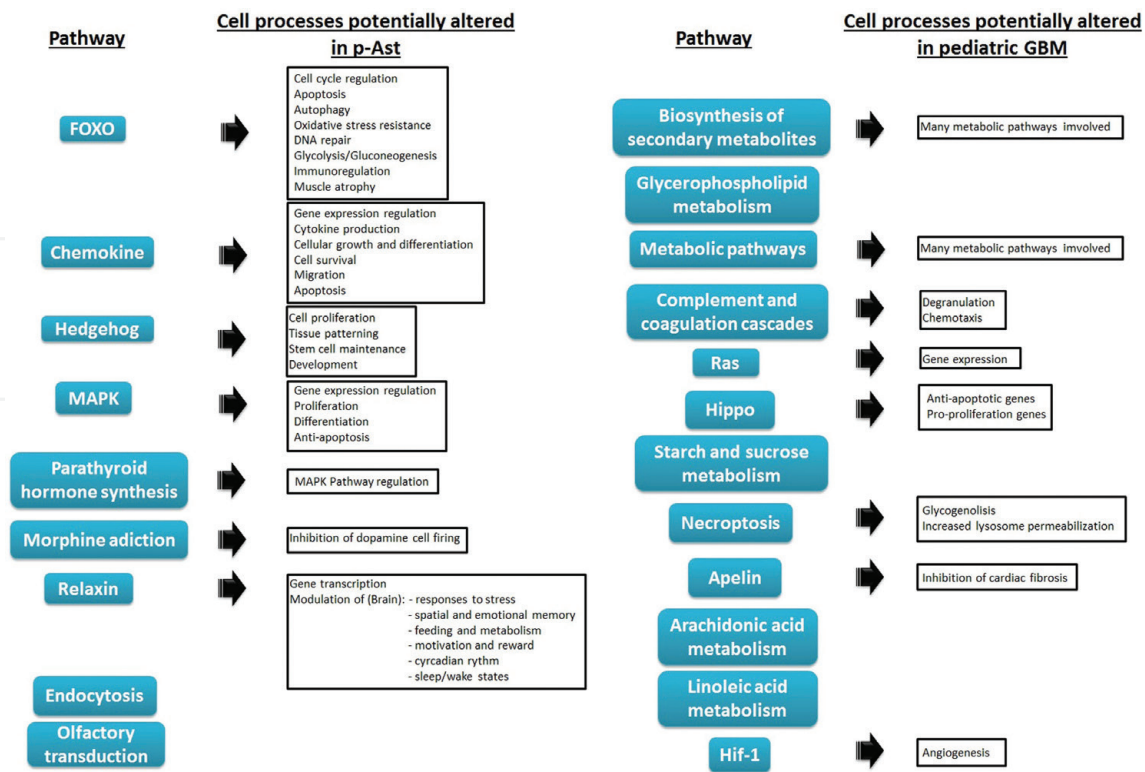


Figure 4. Predicted interaction networks between lncRNAs and mRNAs were predicted to be involved in the control of signaling pathways. Data showed hub mRNAs that were analyzed with the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. Hub mRNAs were those mRNAs with the highest number of interactions with lncRNAs. Data were taken from [5] and analyzed with KEGG.

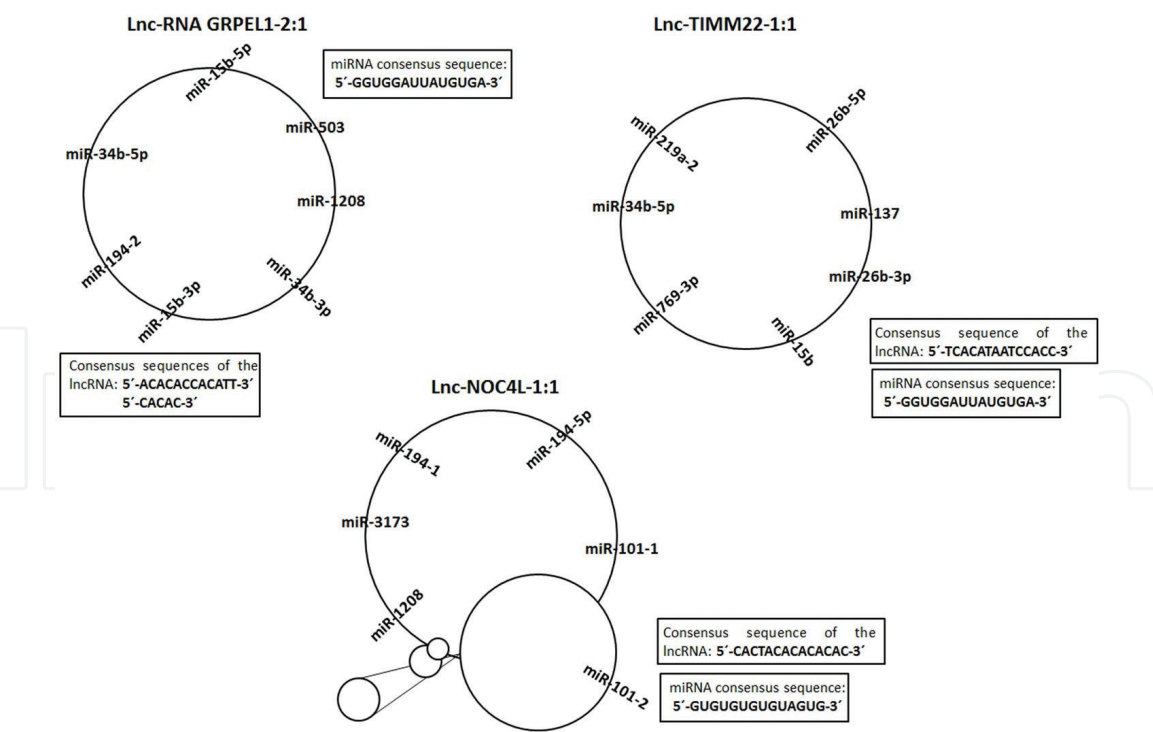


Figure 5.
Circular lncRNAs in pediatric astrocytoma. Predictions showed differentially expressed circRNAs in pediatric astrocytoma, which have many binding sites for miRNAs.

of lncRNAs was predicted to be involved in regulating cellular growth, survival, migration, invasion, adhesion, among others [5] (**Table 5**).

The integration of proteome and mirnome, as well as transcriptome data showed a convergence of all these biomolecules in the control of common signaling pathways, which gave an overview of the action of complex networks in cancer, particularly p-Ast [5, 47]. For example, although it is widely known that the MAPK pathway is altered in ~88% of gliomas, these data showed novel molecular components involved in this signaling pathway in p-Ast, which also allow to differentiate GBM from the other histological grades. The lncRNA *GRPEL1-1:1* was aberrantly expressed in all p-Ast grades when compared to the control tissues, but it was downregulated in WHO grades I–III relative to GBM. It is noteworthy to add, this lncRNA was predicted to interact with miR-15b-5p, and its expression levels were inversely correlated to those of *lnc-GRPEL1-1:1* (**Figure 6**). Other lncRNAs such as *TIMM22-1:1*, *Noc4L-1:1*, and *LINC-ROR* were predicted to be involved in the MAPK pathway, as well as in the Wnt pathway and extracellular matrix interactions [5]. In pediatric GBM, the overexpression of linc-Ror could lead to the downregulation of miR-145, since there is evidence indicating that linc-Ror sponges to miR-145, which was associated with cancer malignancy [100, 101]. According to our model, the linc-Ror•miR-145 axis could be increasing the expression of *IGFR1* (insulin-like growth factor 1 receptor), *c-Myc* (MYC proto-oncogene, BHLH transcription factor), and *STAT1* (signal transducer and activator of transcription 1), which causes a sustained angiogenesis and increased cell proliferation; however, this must be tested (**Figure 6**). In patients with glioma, linc-Ror was downregulated and this correlated positively and negatively with the expression of *SOX11* (SRY-box 11) and *KFL4* (Kruppel-like factor 4), respectively [101]. In the GBM cell line U87, *in vitro* assays showed the involvement of this lncRNA in the induction of cell proliferation, *CD133* expression, and in the formation of neurospheres [101], which are the factors of tumor malignancy. Similarly, linc-Ror was downregulated in p-Ast grades I–III, but it was upregulated in GBM relative to control tissues and other p-Ast grades [5]. Therefore, linc-Ror seems to be a candidate to function as a biomarker for p-Ast diagnosis and prognosis.

KEGG pathway	p-value	Number of genes	Number of miRNAs	Potential cellular processes altered
Proteoglycans in cancer	8.91e-11	120	14	Cellular growth and survival Cell migration and invasion Cell adhesion Apoptosis Angiogenesis Vascular permeability
Fatty acid metabolism	9.64e-09	28	12	Fatty acid metabolism
Adherens junction	3.85e-08	49	12	Actin polymerization Cell growth and differentiation Gene expression
Cell cycle	3.85e-08	85	14	Ubiquitin mediated proteolysis DNA biosynthesis Origin recognition complex Mini-Chromosome maintenance
Protein processing in the endoplasmic reticulum	2.59e-07	101	14	Proteasome Apoptosis
Fatty acid elongation	1.78e-06	13	7	Fatty acid degradation Fatty acid biosynthesis
p53 signaling pathway	2.12e-06	50	14	Cell cycle arrest Apoptosis Inhibition of angiogenesis and metastasis DNA repair and damage prevention Inhibition of IGF-1/mTOR pathway Exosome mediated secretion p53 negative feedback Cellular senescence
Hippo signaling pathway	2.29e-06	77	14	Pro-apoptotic genes Anti-apoptotic genes Pro-proliferation genes Cell contact inhibition Organ size control Adherens junctions
TGF-beta signaling pathway	2.32e-06	48	12	Differentiation, neurogenesis, ventral mesoderm specification Angiogenesis, extracellular matrix neogenesis, immunosuppression, apoptosis induction. G1 arrest Gonadal growth, embryo differentiation, placenta formation Left-right axis determination
Prion diseases	9.44e-06	15	9	Neuronal apoptosis Autophagy Oxidative stress Proliferation of astrocytes

Table 5.
Pathways potentially regulated by differentially expressed super sponges in pediatric astrocytoma; DIANA MirPath V 3.0 analysis.

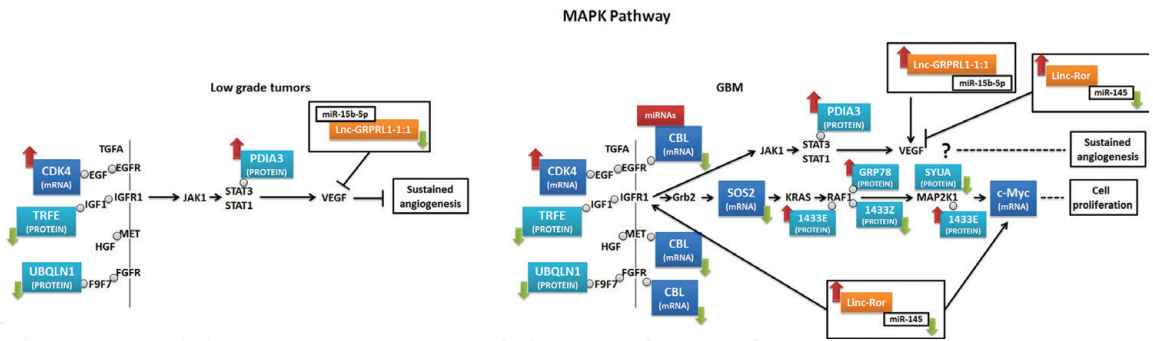


Figure 6. LncRNAs were predicted to be involved in the control of signaling pathways. Differentially expressed lncRNAs were involved in controlling many signaling pathways by interacting with both mRNAs and miRNAs. Further experimental validation is necessary.

6. Conclusions

The lncRNA study in Ast has demonstrated an aberrant expression of this type of RNAs in both tumors and blood, which was useful to distinguish Ast from its nonneoplastic counterpart. The elucidation of molecular signatures from circulating lncRNAs is very promising due to their potential use as noninvasive tools for the diagnosis and prognosis of Ast. From another approach, it could be relevant the identification of complete interaction networks in which lncRNAs, other RNA species, and proteins were involved, since this would give a “panoramic vision” of how the aberrant system functions in astrocytic tumors. This could be crucial for the creation of molecular tools for their treatment.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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